Niobium & Vanadium Quantum Dot and their application in Cell Imaging

Vivek Pratap Singh

Department of Biotechnology and Medical Engineering
National Institute of Technology Rourkela

Niobium & Vanadium Quantum Dot and their application in Cell Imaging

Dissertation submitted in partial fulfillment

of the requirements of the degree of

Master of Technology

in

Biomedical Engineering

by

Vivek Pratap Singh
(Roll Number: 215BM1444)

based on research carried out

under the supervision of

Prof. Sirsendu Sekhar Ray

May, 2017

Department of Biotechnology and Medical Engineering
National Institute of Technology Rourkela

Department of Biotechnology and Medical Engineering
National Institute of Technology Rourkela

May 31, 2017

Dr. Sirsendu Sekhar Ray

Assistant Professor

Supervisors' Certificate

This is to certify that the work presented in the dissertation entitled **"Niobium & Vanadium Quantum Dots and their application in Cell Imaging"** submitted by *VIVEK PRATAP SINGH* Roll Number 215BM1444, is a record of original research carried out by him under our supervision and guidance in partial fulfilment of the requirements of the degree of *M.Tech. Biomedical Engineering* in *Department of Biotechnology and Medical Engineering*. Neither this dissertation nor any part of it has been submitted earlier for any degree or diploma to any institute or university in India or abroad.

Dr. Sirsendu Sekhar Ray

Department of Biotechnology and Medical Engineering

National Institute of Technology, Rourkela

Dedication

To my loving Family

Declaration of Originality

I, Vivek Pratap Singh, 215BM1444, hereby declare that this dissertation entitled **"Niobium & Vanadium Quantum Dots and their application in Cell Imaging"** represents my original work carried out as a postgraduate student of NIT Rourkela and, to the best of my knowledge, it contains no material previously published or written by another person, nor any material presented for the award of any other degree or diploma of NIT Rourkela or any other institution. Any contribution made to this research by others, with whom I have worked at NIT Rourkela or elsewhere, is explicitly acknowledged in the dissertation. Works of other authors cited in this dissertation have been duly acknowledged under the section "Bibliography". I have also submitted my original research records to the scrutiny committee for evaluation of my dissertation.

I am fully aware that in case of any non-compliance detected in future, the Senate of NIT Rourkela may withdraw the degree awarded to me on the basis of the present dissertation.

May 31, 2017 Vivek Pratap Singh

NIT Rourkela

Acknowledgment

First of all, I would like to thank **God,** for his blessing that enabled me to complete my project work. I would like to convey my deep gratitude to my parents and my other family members for their blessing, encouragement, love, and support.

I would like to express my heartfelt gratitude to my Supervisor **Prof. Sirsendu Sekhar Ray**, Department of Biotechnology & Medical Engineering, for giving me the opportunity to do this project work and for his helpful suggestion, encouragement, support, and blessing.

I am highly grateful to **Prof. Mukesh Kumar Gupta**, H.O.D, and Department of Biotechnology & Medical Engineering, for their support and providing me lab facilities available in the department.

I am thankful to my lab members and my classmates for their love and support.

Vivek Pratap Singh

Abstract

Niobium oxide nanoparticles & quantum dot and Vanadium quantum dot were synthesized by sol-gel method. Niobium pentoxide was solubilized with hydrofluoric acid in order to get niobic acid precipitate by using ammonia solution. To reduce the particle size, the niobic acid was treated with hydrofluoric acid. Vanadium pentoxide was solubilized with hydrogen peroxide and hydrothermally treated to get Vanadium oxide quantum dot, but instead of getting quantum dot we got layered structure of vanadium oxide. The samples were characterized by TEM, FE-SEM, SEM XRD and FTIR. From these characterization it was observed that the average size of the niobium oxide nanoparticles was found to be 30nm. The size, shape, morphology and surface area of the nanoparticles are highly dependent upon the amount of ammonia solution used for their synthesis, temperature and duration of hydrothermal treatment that they are subjected to. The cell imaging of niobium oxide nanoparticle and diluted vanadium oxide ultra-sonicated gel was done by confocal microscopy which showed fluorescence. Supercapacitor was also developed by niobium oxide nanoparticle.

Keywords: ***Niobium& Vanadium quantum dot, Sol-gel method, hydrothermal treatment, cell imaging, super capacitor***

Contents

Supervisors' Certificate iii

n**Dedicationn** iv

Declaration of Originality v

Acknowledgment vi

Abstract vii

Introduction 13

Literature review 16

Materials and Methods 20

3.1 Synthesis process of metal oxide nanoparticles 20

3.1.1 Materials 20

3.1.2 Synthesis of Niobium oxide nanoparticles & Quantum dot 20

3.1.3 Syntesis of Vanadium Oxide Quantum dot 21

3.2 Characterization of nanoparticles - 21

3.2.1 FE-SEM (Field emission scanning electron microscopy) 21

3.2.2 TEM (Transmission electron microscope) 22

3.2.3 FTIR (Fourier Transform Infrared Spectroscopy) 22

3.2.4 XRD (X-ray Diffraction) 22

3.2.5 Cytotoxicity assay (MTT assay) 23

3.2.6. Spectro-fluorimetry 23

Results and Discussion 24

Part - I 24

4.1 FE-SEM (Field emission scanning electron microscopy) - 24

4.2 Elemental analysis, EDAX (Energy Dispersive X-ray) 25

4.3 TEM (Transmission electron microscope)- 25
4.4 HAADAF (High Angle Angular Dark Field) STEM 27
4.5 XRD (X-ray Diffraction) 28
4.6 FTIR (Fourier Transform Infrared Spectroscopy) 29
4.7 In-Vitro Cytotoxicity assay 30
4.8 Cell Imaging- 30
4.9 Spectrofluorimetry 31
4.10 SUPER CAPACITOR 32
Part - II 37
4.11 FE-SEM (Field emission scanning electron microscopy 37
4.12 TEM ((Transmission electron microscope)- 38
4.13 HAADAF (High Angle Angular Dark Field) STEM 39
4.14 XRD (X-ray Diffraction) - 40
4.15 Cell Imaging 41
Conclusion 42
References 43

List of figures

Figure 4. 1 FESEM images of (a,b) Niobium Pentoxide powder, (c,d) Niobium nanoparticles.. 24

Figure 4. 2 EDAX pattern of SEM image of niobium oxide nanoparticle 25

Figure 4. 3: (a),(b) & (c)TEM images of niobium oxide nanoparticles (d) SAED pattern of niobium oxide nanoparticles 26

Figure 4. 4: TEM images of niobium oxide quantum dot..... 27

Figure 4. 5: HAADF images of niobium nanoparticles 28

Figure 4. 6: XRD patterns of the (a) Precursor of Niobium Pentoxide (b) Niobium oxide nanoparticles 28

Figure 4. 7: FTIR spectra of (a) Niobium Pentoxide powder (b) Niobium oxide quantum dot .. 29

Figure 4. 8: Invitro cytotoxicity assay of Niobium nanoparticles 30

Figure 4. 9: Confocal microscopy images of HeLa cell stained with niobium oxide quantum dot 31

Figure 4. 10: Spectrofluorimetry spectra of niobium oxide nanoparticle 31

Figure 4. 11:Steps involved in making Supercapacitor 33

Figure 4. 12 :Niobium-Charcoal-Sodium Sulphate Supercapacitor 34

Figure 4. 13 : Niobium-Charcoal-KOH Supercapacitor 34

Figure 4. 14 Charcoal-Charcoal sodium sulphate Supercapacitor 35

Figure 4. 15 Niobium-Niobium-Sodium sulphate Supercapacitor 35

Figure 4. 16 Charcoal-Charcoal- KOH supercapacitor..... 36

Figure 4. 17 : Niobium-Niobium-KOH Supercapacitor 36

Figure 4. 18: FESEM images of (a) Vanadium Pentoxide powder, (b,c,d) Vanadium layered ... 37

Figure 4. 19: TEM images of Vanadium oxide layer 38

Figure 4. 20: HAADF image of Vanadium oxide layer..... 39

Figure 4. 21: XRD Pattern of Vanadium pentoxide powder(a) vanadium oxide layer H_2O_2(b) vanadium oxide layer H_2O..... 40

Figure 4. 22 Confocal microscopy images of HeLa cell stained with vanadium oxide quantum dot 41

LIST OF ACRONYMS AND ABBREVIATIONS

QD	Quantum dots
MQDs	Mica Quantum Dots
NQDs	Niobium Quantum Dots
NP	Niobium Pentoxide
SEM	Scanning Electron Microscope
FE-SEM	Field emission scanning electron microscopy
TEM	Transmission electron microscope
STEM	Scanning Transmission electron microscope
HRTEM	High resolution Transmission electron microscope
HAADF	High angle angular dark field
EDAX	Energy dispersive X-ray
XRD	X-ray Diffraction
FTIR	Fourier Transform Infrared Spectroscopy
SAED	Selected Area Electron Diffraction
TCP	Tissue Culture Plate

Chapter 1

Introduction

The view of nanotechnology however considered a present science has its records going back in the Mesopotamian time is as yet exploited for further conceivable outcomes. The synthesis of metallic nanoparticles is a dynamic area of academic and application research in nanotechnology . The proliferation of nanotechnology has opened up novel central and applied frontiers in materials science and engineering in the past couple of decades. The idea of nanotechnology was given by Nobel Laureate Physicist Richard Feynman, in 1959 (Patra et al., 2013). "Nano" is a Greek word synonymous to dwarf meaning to an extremely small. The term Nanotechnology (once in a whi e abbreviated to "nanotech") originates from nanometer – a unit of estimation of one billionth of a meter of length. Nanotechnology manages structures that range between 1 to 100 nanometers in no ess than one measurement and includes creating materials or devices having no less than one measurement inside that size (Ahmad et al., 2003). In spite of the fact that the idea of a basic synthesis of nanoparticles continues as before i.e. Top down technique and Bottom-up approach , in this day and age, nanotechnology is a various field , ranging from extension of traditiona devices to totally new methodologies in light of atomic assembly. It is likewise an amalgamating innovation which has fascinating multi-disciplinary application in different aspects of biosensors medication, biological, beautifying cosmetics , sustainable power sources, ecological remediation and biomedical devices (Tran et al., 2013). Even though different chemical and physical procedures are at present broadly in used to synthesize metal nanoparticles, enabling one to acquire particles with the required qualities (Asim et al., 2012), chemical procedures are a the more frequently implied for the synthesis of nanoparticles. While the investigation of nanometer-scale materials has been in presence for a long while now, advances in synthesis and characterization methods have catalyzed the immense development in nanoscience over the recent decades. Indeed, even as many items incorporating nanotechnology are commercialized, the comprehension of these novel materials is small contrasted with practically equivalent to mass species.

Nowadays energy demand is increasing day by day in the worldwide. With the development of nanotechnology, nanomaterials can be used as a storage element. Quantum dots are the semiconductor nanocrystal materials. Due to the effect of quantum confinement, it exhibits new properties compared to the parent material. However, some properties of parent material are still present. Compared to their normal particle a quantum dot also offers large surface to volume ratio and better solubility in nonaqueous and aqueous solution and the properties can be changed widely by doping of other material. There are many quantum dots have been reported till now e.g. carbon quantum dot, graphene quantum dot, cadmium free quantum dots etc. Quantum dots have wide application in many fields such as biophotonic, Nanomedicine, Energy storage, Pharmacokinetics, LEDs, and display.In this dissertation, we are presenting niobium and vanadium based quantum dot. Niobium is a group V transitional element and found mainly in oxide form. Niobium and oxygen mainly occur in stoichiometric oxide forms such as NbO, Nb_2O_3, NbO_2 and Nb_2O_5. In which niobium pentoxide (Nb_2O_5) is widely known and studied material. Niobium pentoxide is an inorganic and constituent of ferroelectric compound. Niobium pentoxide gives N- Type semiconductor material property and has energy band gap around 3.4 eV. The structure of niobium pentoxide exist in many polymorphic forms such as TT- Nb_2O_5 shows pseudohexagonal structure, T-Nb_2O_5 shows orthorhombic structure and H- Nb_2O_5 shows monoclinic structure these are the common phases of Nb_2O_5. In these phases H-phase is highly thermodynamic stable structure and TT-phase is the least stable structure. TT- phase can be converted easily into H-phase by appropriate heat treatment technique. In synthesis of niobium nanoparticle first niobic acid formed which shows the amorphous structure and can be converted into TT- Nb_2O_5 phase at lower temperature. TT- Nb_2O_5 is the phase which changes almost continuously into T- Nb_2O_5 on being heated at 600^o C to 900^o C. While if heat treatment is carried out at about 1100^o C of any phase of Nb_2O_5 it can be converted into H-Nb_2O_5. Niobium oxide nanoparticle shows high surface area and high catalytic activity.

There are two methods for synthesis of the quantum dot. The synthesis of Quantum dots through ‘‘bottom-up’’ approach which is more familiar to chemists: molecular or ionic precursors to the quantum dots are allowed to react together in solution to produce the quantum dot materials as colloids. The other approach, more familiar to engineers, is the ‘‘top-down’’

approach: feature sizes on the 1–10 nm scale are carved out lithographically or electrochemically from a semiconductor substrate. Since the discovery of carbon nanotubes, an entirely new field of scientific study relating to the synthesis and characterization of novel inorganic nanostructures has emerged. Niobium oxide and Vanadium oxide are two of the most promising candidates for creating of novel nano structures due to their unique properties. Vanadium nanotubes can be used as a materials for the preparation of electrochemical devices and as a catalyst in catalytic reactions, and it is especially used for dehydrogenations or partial oxidations of alkanes to olefins, and even among the various Magneli phases of vanadium oxide, VO2 has gained researcher focus due to the reversible electrical, optical and magnetic properties at temperatures around 70°C. Niobium oxide is one of the important component of ferroelectric compounds which also have piezoelectric properties ,thus it has an extensive application in creation of actuators and resonators (Koohestani, Tajdari, & Ebrahimi, 2015).

The synthesis of VOx-NT initially used carbon nanotubes as templates, currently there are many choices available, which are structure directing template based on hydrothermal treatment Vanadium oxide nanotubes can be synthesized with high yield and verities of unique structure like as multilayered scrolls, where we can control the distance between the interlayer. The reaction occurs during the preparation of vanadium oxide nanotubes is totally dependent upon the certain factors such as heating temp., appropriate pH and the time duration required for the hydrothermal treatment. The preparation of Vanadium oxide nanotubes can be accomplished in three ways: (1) Adding a template to vanadium oxide gels followed by the hydrothermal treatment; (2) Grinding a mixture of the template and vanadium oxide; and (3) Melt quenching (Koohestani et al., 2015)

Chemical sensors are nothing but a analytical devices, which can information about the chemical component present in the environment and if any biological component (cells, tissue, enzyme etc) associated with that analytical devices than that type of sensor is known as biological sensor.

Vanadium and Niobium nanoparticles have unique physiochemical properties and also it is biocompatible, cytotoxic, and antioxidative in nature.These nanoparticles also posses photoluminescence activity. These nanoparticles therefore can act as a good chemical and biological sensor, which can bind with a specific target analyte.

Chapter 2

Literature review

Porous Nb_2O_5 nanoparticles were synthesized by treating niobium oxide powder with hydrofluoric acid and pH was maintained by adding ammonia solution. At pH 9 due to peptization reaction niobic acid precipitate was formed. The precipitate was filtered and washed and then dried in vacuum oven at 80ºC. The amorphous powder was than calcined 580º for 30min. After calcinations porous Nb_2O_5 nanoparticles was obtained. The synthesized nanoparticles are characterized by XRD, TEM, SEM, and BET analyzer. The average pore size of the resulted Nb_2O_5 nanoparticles are around 3.4nm (Zhou, Qiu, L??, Zhang, & Ma, 2008).

Niobium oxide nanoparticles were synthesized from peroxo niobic acid sol by low temperature synthesis method. Niobic acid precipitate was obtained by treating niobium Chloride in EtOH solution and pH was adjusted by adding ammonia solution. Peroxo niobic acid sol was obtained by treating niobic acid precipitate with Hydrogen peroxide solution. By heating peroxo niobic acid sol we can get Niobium nanoparticles and niobic acid nanoparticles. The obtained nanoparticles were characterized by FTIR, XRD, BET, TG, and FESEM analysis. The resulted Niobium oxide nanoparticles size is around 4.5nm and niobic acid nanoparticles size are less than 2nm in diameter (Uekawa et al., 2003).

Niobium oxide nanorods are synthesized by hydrothermal treatment and these nanorods are deposited on to the surface of the graphite felt electrode in all vanadium flow battery. Niobium oxide also can be used as catalyst and it can enhance electrode. By decorating nanotubes with graphite felt can enhance the electrode performance for vanadium flow battery (Access, 2016).

Vanadium oxide nanotubes were synthesized by treating vanadium pentoxide with hydrogen peroxide. Due to exothermic reaction occurs and hydrogen peroxide decomposition one red color gel was obtained. Hexadecylamine solution was prepared with

acetone and added to the red gel and kept for stirring for 16hrs. After stirring the obtained solution was then put at in a parr Teflon digestion bomb for hydrothermal treatment. After hydrothermal treatment obtained sample was washed with acetone and cyclohexane and then dried under oven at 90ºC to get vanadium oxide nanotubes. The resulted powder was characterized by XRD, TEM and SEM (Koohestani et a ., 2015).

Vanadium Pentoxide was treated with hydrogen peroxide. The reaction was then kept for stirring. Due to exothermic reaction occurs, after 10min of stirring orange color solution was formed which become red color gel after 24hrs. About 2.66g of hexadecylamine powder was prepared with 4ml ethanol and put in to that solution as v: amine ratio should be 2:1. The obtained brown color mixture was kept for stirring for about 14hrs.The solution gradually become green color when its pH fall to 4.5.The obtained sample was then washed with cyclohexane and ethanol and then dried under vacuum oven at 80˚C for 5hrs to get Vanadium oxide nanotubes. The sample was characterised by TEM, SEM, XRD, TGA (Kianfar, 2015).

Vanadium oxide nanotubes were prepared from vanadium oxytrichloride. Around 7mmol of vanadium oxytrichloride was treated with primary in a molar ratio of 2:1 and the solution was kept for stirring in an inert atmosphere. After aging the sample was hydrolyzed with acetate buffer at 0ºC. One green solution obtained. After aging of 24hrs the solution became brown color and was filtered and washed. The sample was than treated with isopropanol kept for hydrothermal treatment in a Teflon autoclave at 180ºC for 7days. The resulted powder was than washed with ethanol and hexane and dried in vacuum oven to get nanotubes. The sample was characterized by TEM and XRD (Niederberger et a ., 2000).

Vanadium oxide was treated with methanol and then this solution was poured drop by drop to the solution containing 35% hydrogen peroxide solution and stirred for 10min under room temperature. The solution was stirred for 1hr. After 1hr of stirring the solution was then hydrothermally treated by autoclaved at Teflon autoclave at 150ºC for 24hrs. The obtained sample was than dried under vacuum oven at 60ºC for 12hrs. The

precursor was than calcined at 400ºC for 2hr in air to obtain vanadium Pentoxide hollow sphere. The sample was than characterized by XPS, XRD, TEM, and FTIR (Wu et a ., 2016).

For the synthesis of silver nanoparticles used a solution of silver nitrate ($AgNO_3$)and water/ethanol and boiled in an ambient atmosphere and 1% tri sodium citrate solution. Silver nitrate solution was then kept on a hot plate at 90ºC for 5 minutes and then trisodium citrate was added drop by drop once the reduction process begins colour change appears and the solution turn into pale yellow. After the changes in colour, the solution was stirred in magnetic stirrer for 15 mins. The characterization of silver nanoparticles was done by UV-visible spectroscopy and scanning tunneling microscope (Jaiswa et a ., 2003).

$AuCl_4 33H_2O$ and TOABr was used for the synthesis of Au nanoclusters by mixing with methanol. After being vigorously stirring the solution color changes from yellow to dark red. Then , $C_6H_{13}SH$ or thiols was added to the solution at room temperature; the color of reaction mixture rapidly turns white. A fresh $NaBH_4$ solution was rapidly added to the solution with continuous stirring. The color of the solution immediately turns black and produces Au clusters, which are then precipitated out of the methanol solution through centrifugation at 5000 rpm. The synthesis nanoclusters are characterized by UV-visible spectroscopy , MELDI mass spectroscopy, time of flight (TOF) mass spectroscopy (Mishra et a ., 2012).

$H_2PtC_6H_2O$ metal ions transferred into a toluene solution by a phase transformation process and synthesized nanoparticles using two different phases. In Phase, I an aqueous solution of Pt precursor was mixed with tetra octyl ammonium bromide (TOAB) by vigorously stirring for 15min. The organic phase was then separated. In 2nd phase copper acetate and Hexa decy -trimethyl ammonium bromide (CTAB) were added into toluene, and the mixture was heated at 120°C under magnetic stirring to form a dark green solution. Afterward, a freshly prepared sodium borohydride ($NaBH_4$) was added with vigorously stirring. The dark green solution changed into dark brown within a minute indicating the

formation of Cu nano particles. Then, dodecanethio was added as stabilizing agent. The colloida solution was left for cooling at room temperature, and the product was redispersed in organic solution (Khana et a ., 2014).

Chapter 3

Materials and Methods

3.1 Synthesis process of metal oxide nanoparticles

3.1.1 Materials

Different chemicals used in these experiments are Niobium Pentoxide, Hydrofluoric acid, ammonia solution, ethanol, and hydrogen Peroxide and for synthesis of Vanadium oxide quantum dot Vanadium Pentoxide, ethanol, and hydrogen Peroxide required. All chemicals was purchased from HIMEDIA. Different equipments also used in this synthesis process are Hot water bath, Centrifuge, Hot air oven, Teflon coated autoclave, Furnace. All equipments are purchased from good quality supplier.

3.1.2 Synthesis of Niobium oxide nanoparticles & Quantum dot

The niobium Pentoxide nanoparticles and the niobic acid nanoparticles were synthesized as follows. Commercial Niobium Pentoxide powder was treated with hydrofluoric acid at a concentration of 20g/l. The solution was then heated in water bath at a temperature between 80ºC to 100ºC and slowly ammonia was added to the solution to maintain its pH. At a pH 9, white colour precipitate niobic acid (Nb 2O 5·n H $_2$O) was formed. Excess fluoride ion was removed by washing with ethanol and distilled water. The white precipitate was separated by centrifugation at 13500 rpm for 15min. Niobic acid was washed with milli Q water to remove all impurities followed by repetitive centrifugation. The niobic acid was then dried at 60ºC in a hot air oven for 12hrs to obtain niobium nanoparticles. The obtained powder was niobium oxide nanoparticle. To obtain the niobium oxide quantum dot, nanoparticle was dispersed in milli Q water by ultra-sonication for 30 min. The resultant solution was centrifuged at 13500 rpm to separate supernatant and pellet. Supernatant contain niobium oxide quantum dot.

3.1.3 Syntesis of Vanadium Oxide Quantum dot

(a) Vanadium oxide layer (H_2O_2) -

Vanadium oxide layer was prepared by hydrothermal treatment. 500 mg. of crystalline V_2O_5 was added to 20 ml hydrogen peroxide (H_2O_2), which was 30% by weight. During synthesis of quantum dots, exothermic reactions occur which lead to the partial decomposition of hydrogen peroxide due to which release of oxygen occurs and peroxo complexes form. Clear orange solution was started forming after about 10 min of stirring that gradually turns into a red colour viscous gel after 24 hr. of stirring. This viscous gel has been correspond to V_2O_5 .nH_2O, where the amount of water depends on the experimental conditions. In this viscous gel 10ml. additional H_2O_2 was added. Resulted mixture was kept for hydrothermal treatment at 160^o C for 6 hr. After that, the vanadium oxide gel was dried at 60^{0}C which was converted into red film.

(b) Vanadium oxide layer (H_2O) –

Vanadium oxide layer was prepared by hydrothermal treatment. 500 mg. of crystalline V_2O_5 was added to 20 ml hydrogen peroxide (H_2O_2), which was 30% by weight. During synthesis of quantum dots, exothermic reactions occur which lead to the partial decomposition of hydrogen peroxide due to which release of oxygen occurs and peroxo complexes form. Clear orange solution was started forming after about 10 min of stirring that gradually turns into a red colour viscous gel after 24 hr. of stirring. This viscous gel has been correspond to V_2O_5 .nH_2O, where the amount of water depends on the experimental conditions. In this viscous gel 10ml. additional H_2O was added. Resulted mixture was kept for hydrothermal treatment at 160^o C for 6 hr. After that, the vanadium oxide gel was dried at 60^{0}C which was converted into light green film.

3.2 Characterization of nanoparticles -

3.2.1 FE-SEM (Field emission scanning electron microscopy)

The morphology and structure of the niobium oxide nanoparticles and Vanadium oxide layers were evaluated by FE-SEM (Nova Nano Sem, FEI).Each specimen was fixed on to the specimen holder by using double sided tap and coated with gold to achieve thickness of

200Aº in a plasma sputter(QS 1050 Quorum Tech.). The appearance and Morphology of the sample were observed by FE-SEM at an accelerating voltage of 10-20KV.

3.2.2 TEM (Transmission electron microscope)

The structure and morphology of the niobium oxide nanoparticles, nibium oxide quantum dots and Vanadium quantum dots were observed under Transmission electron microscopy analysis was carried out by using a JEM-100CX2 transmission electron microscope with high accelerating voltage 100-300KV and also EDX analysis of metal oxide nanostructures was done for elemental analysis.

3.2.3 FTIR (Fourier Transform Infrared Spectroscopy)

Fourier Transform Infrared spectroscopy study of the niobium nanoparticles, niobium quantum dots and Vanadium quantum dots were done in order to analyse the chemical interactions and the bonds present in the sample .For the analysis, FTIR-spectrometer attached with an ATR cell of ZnSe was used (Alpha-E-Brunker, Germany). The niobium oxide nanoparticles and vanadium oxide layers, vanadium quantum dots were analyzed in a wavenumber 500cm^{-1}-4000cm^{-1} range of at a reso ution of 8 cm^{-1} for a tota 24 scans.

3.2.4 XRD (X-ray Diffraction)

X-Ray Diffraction (XRD) is a laboratory-based technique commonly used for identification of crystalline materials and analysis of unit cell dimensions. The crystalline size was calculated using the following Debye–Scherer equation.

$$X_S = \frac{0.9\lambda}{\beta cos\theta}$$

Where , X_S is the crystalline size, λ is the wavelength of the-ray beam, β is the Full Width at Half Maximum (FWHM) of the peak at the maximum intensity and θ is the diffraction peak angle. Niobium oxide nanoparticles and Vanadium oxide layers were analyzed by X-Ray diffraction using Ultima IV Multipurpose XRD Diffractometer (Bruker D8 Advance). The

instrument used an X-Ray source of Cu-Kα, and was operated at a voltage of 40kv and a current of 40 mA. The analysis was done in the 2Θ range of 5º to 80º at a scan rate of 5º (2θ) / min. The ana ysis was performed at the room temperature.

3.2.5 Cytotoxicity assay (MTT assay)

In-vitro cytotoxicity assay of Niobium Quantum Dots (NQDs) and vanadium quantum dots were done with the HeLa cell line (Osteocircoma bone cancerous cell). The cell line was maintained in 96 well plate, 104 cells/well (200µl/well) were seeded and maintained in DMEM containing 10% FBS in CO_2 incubator. MTT was added after 1.3 days of seeding and incubated for 4hrs. After incubation, media was decanted and 200µl of DMSO was added to each well and reading taken at 590nm.

3.2.6. Spectro-fluorimetry

To study the photoluminescence activity and to determine the absorption peaks in a specific wavelength and shifting of absorption peaks in different excitation state of NQDs and Vanadium Quantum dots in aqueous solution spectrum scan was conducted from absorption spectra 200 to 800nm in different excitation wavelength from 200 to 800nm.

Chapter 4

Results and Discussion
Part - I

4.1 FE-SEM (Field emission scanning electron microscopy) -

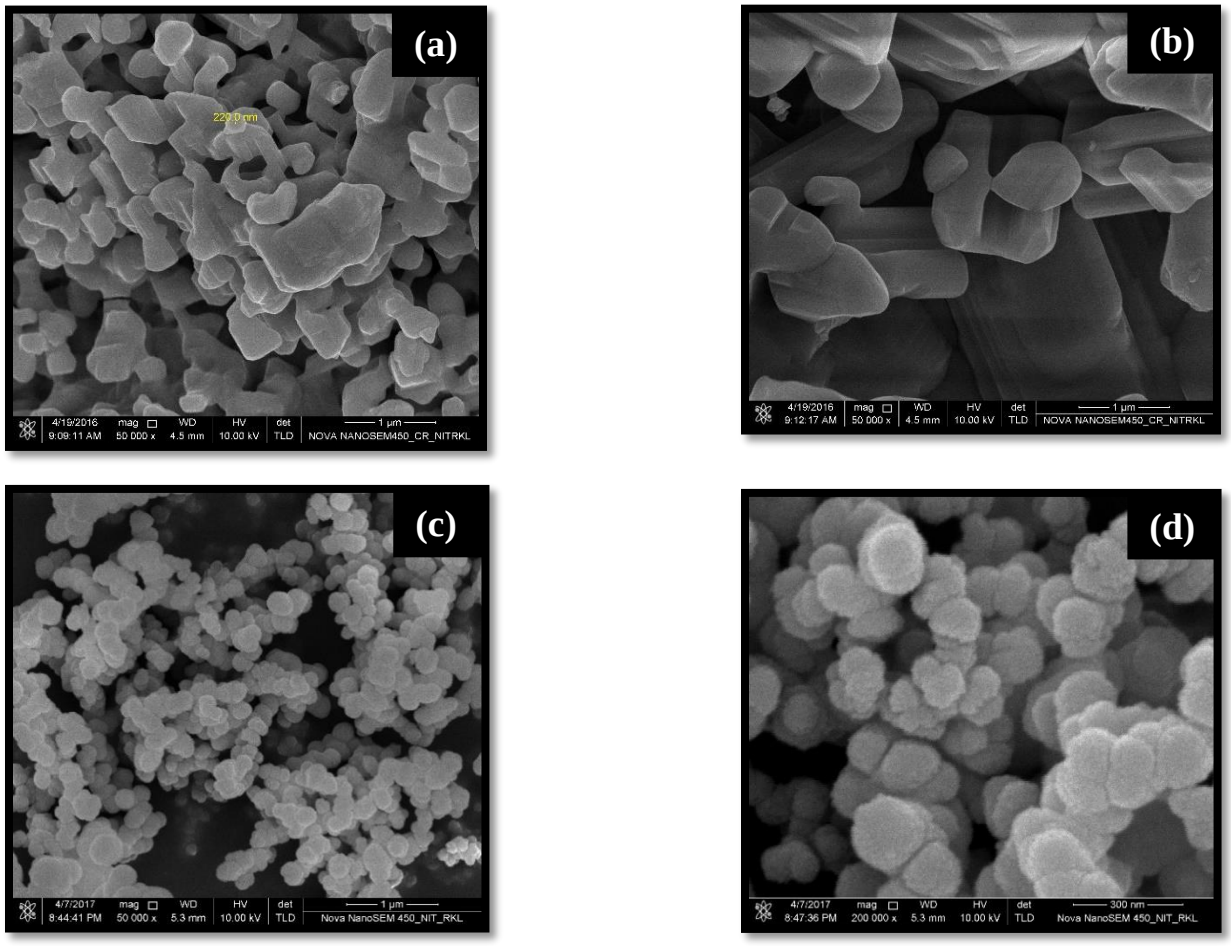


Figure 4. 1 FESEM images of (a,b) Niobium Pentoxide powder, (c,d) Niobium nanoparticles.

The FESEM image of the niobium Pentoxide precursor fig 4.1 (a), (b) shows that the particle size is quite bigger than 300nm or more than that. The niobium oxide nanoparticles in the fig 4.1 (c), (d) shown that the nanoparticles are in aggregated form and are spherical in shape. The average size of the niobium oxide nanoparticles were found around 60 nm ±6.

4.2 Elemental analysis, EDAX (Energy Dispersive X-ray) - EDAX analysis was also done to establish the elemental composition of the synthesized niobium oxide nanoparticle. The EDAX pattern of SEM image of niobium oxide nanoparticles shown in Figure 4.1 (c) and (d) confirms the presence of niobium (Nb), carbon(C) and Oxygen (O) as shown in figure 4.2.

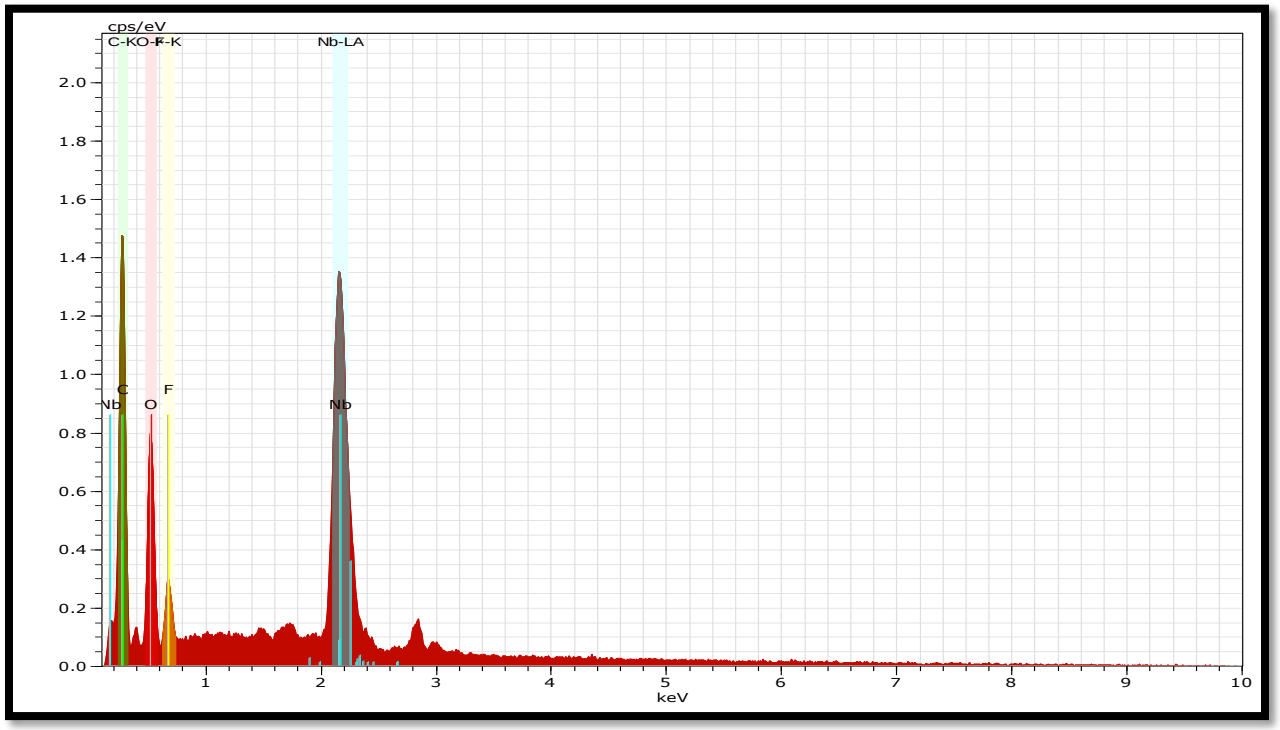


Figure 4. 2 EDAX pattern of SEM image of niobium oxide nanoparticle

4.3 TEM (Transmission electron microscope)- The Transmission Electron Microscope (TEM) images of niobium oxide nanoparticle is shown in Fig. 4.3. It was observed that the niobium oxide nanoparticles are synthesized successfully and are in spherical form as shown in Fig. 4.3(a). It was also observed that some nano particles formed core sell like structure as shown in Fig. 4.3(a) & (b). The synthesized niobium oxide nanoparticle showed amorphous structure which was confirmed by SAED pattern of niobium oxide nanoparticle as shown in Fig 4.3 (d).

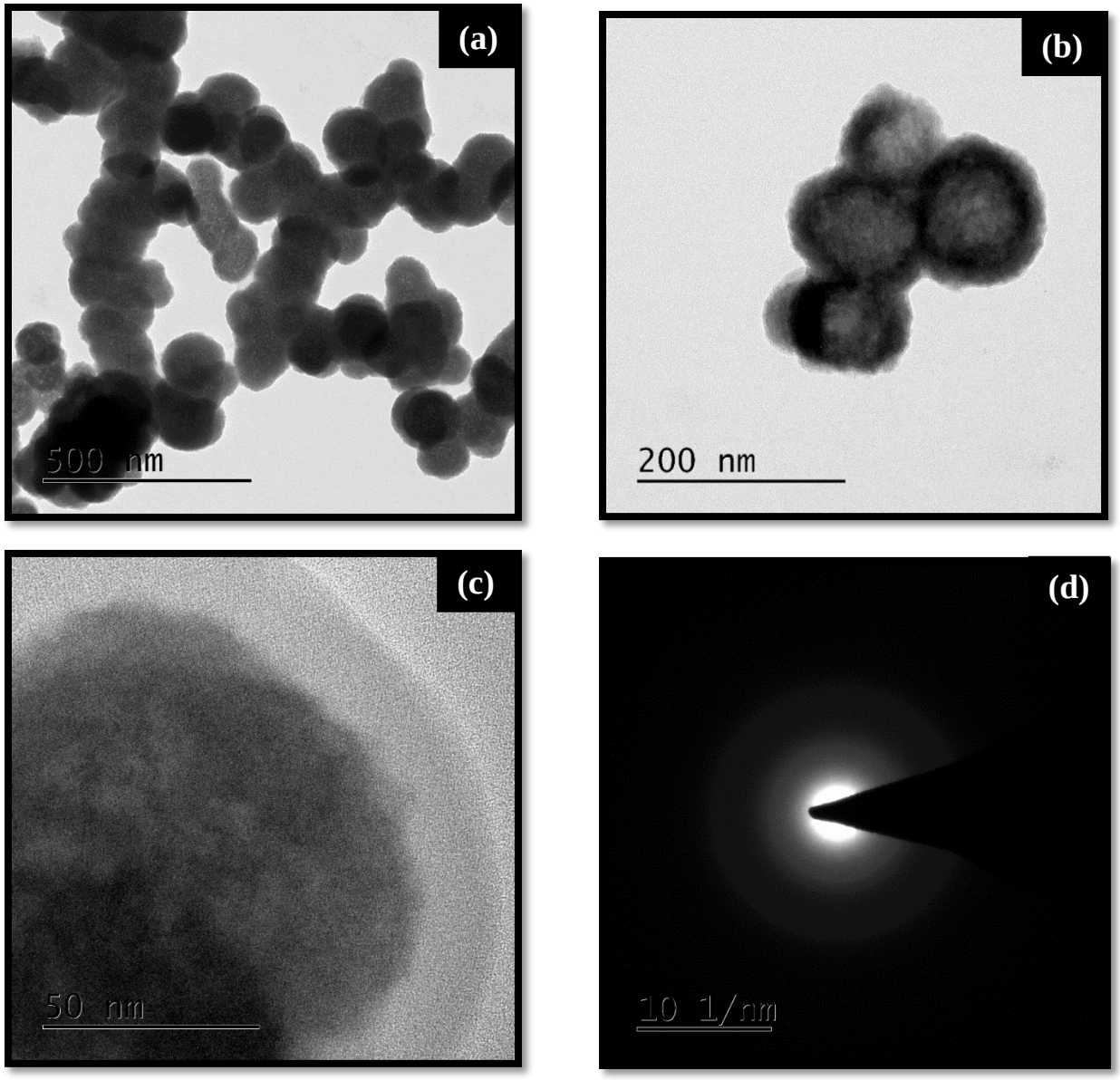


Figure 4. 3: (a),(b) & (c)TEM images of niobium oxide nanoparticles (d) SAED pattern of niobium oxide nanoparticles

Niobium oxide quantum dots were also seen at higher magnification as shown in Fig. 4.4 (a) & (b). The average size of niobium oxide quantum dots were found in the range of 5nm. - 8nm. This smaller size of treated niobium powder confirms the synthesis of Niobium oxide Quantum Dots (NQDs).

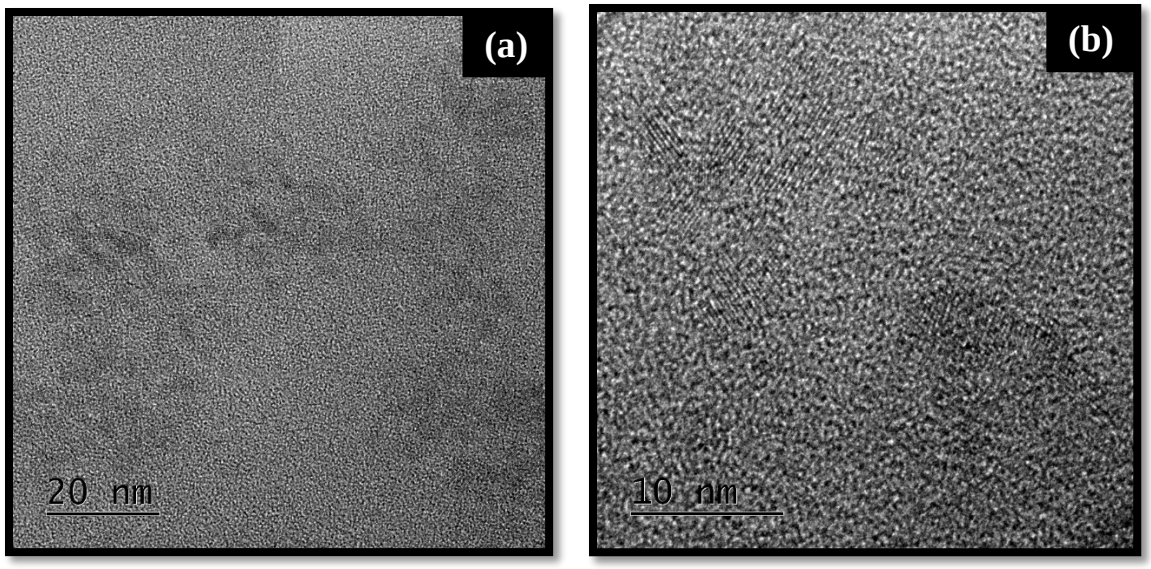
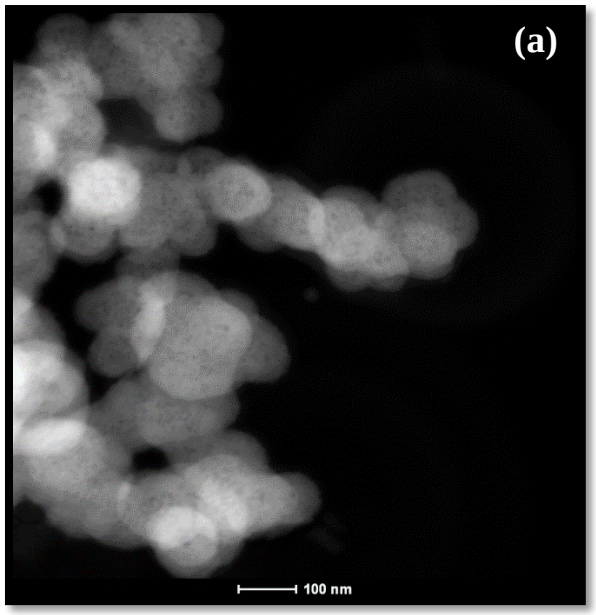


Figure 4. 4: TEM images of niobium oxide quantum dot

4.4 HAADAF (High Angle Angular Dark Field) STEM - The structural distributions of niobium oxide nanoparticles were showed in the Fig. 4.5(a). In STEM mostly spherical structures were appeared. Fig. 4.5 (b), (c) shows the elemental position of Oxygen and Niobium in niobium oxide nanoparticle. The spherical structure of niobium oxide nanoparticles contains mainly oxygen and niobium.



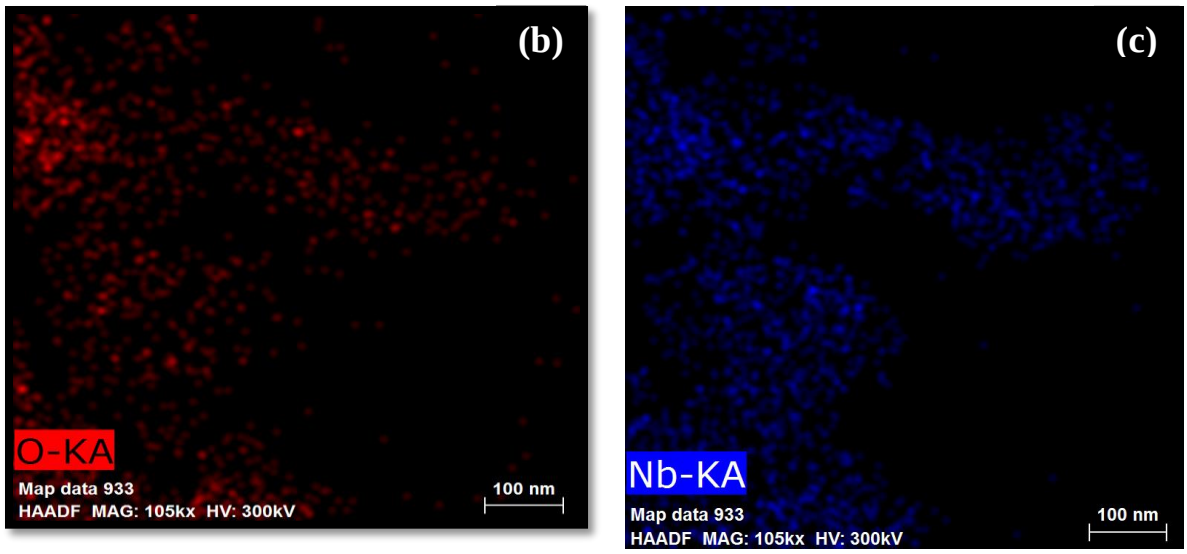


Figure 4. 5: HAADF images of niobium nanoparticles

4.5 XRD (X-ray Diffraction) – XRD pattern of niobium pentoxide powder shows highly crystalline structure as shown in Fig. 4.6(a). While XRD pattern of niobium oxide nanoparticle shows amorphous structure which also support the SAED pattern of it as shown in Fig. 4.6(b). So XRD pattern confirms that synthesized niobium oxide particles were amorphous in structure.

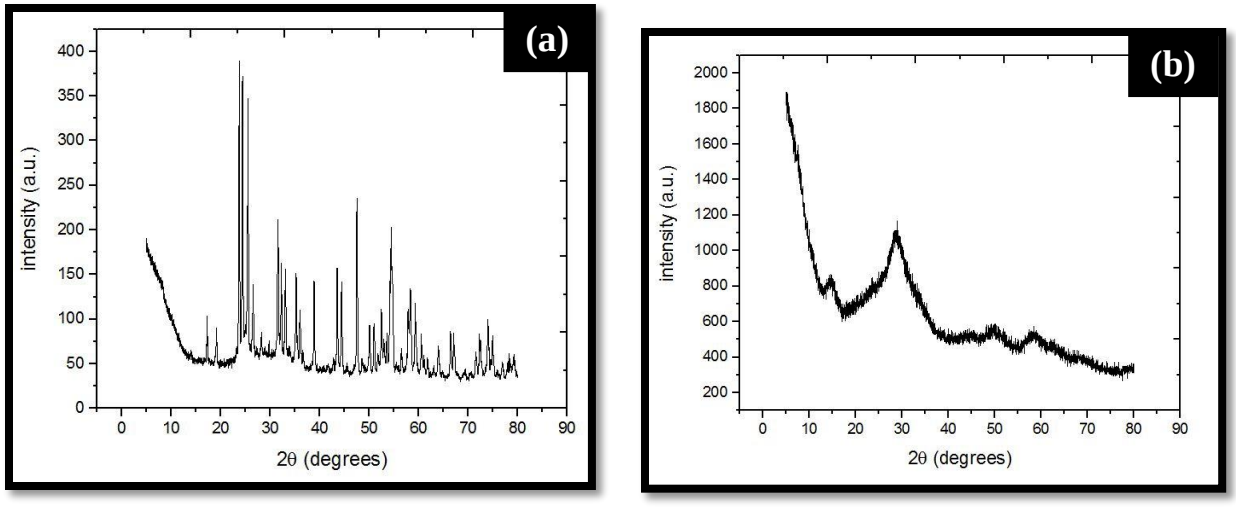


Figure 4. 6: XRD patterns of the (a) Precursor of Niobium Pentoxide (b) Niobium oxide nanoparticles

4.6 FTIR (Fourier Transform Infrared Spectroscopy) – Fig. 4.7 (a) show the FTIR spectra of Niobium Pentoxide powder. The resulted spectrum bands of niobium oxide nanoparticle Fig. 4.7 (b) and niobium quantum dot Fig. 4.7 (c) are the characteristics of amorphous niobium oxide. In the Fig. 4.5 (b) and absorption peak from 3000 cm^{-1} to 3750 cm^{-1} can be observed due to the stretching vibration of the hydrogen-bonded OH groups of the adsorbed water and the niobic acid. The absorption at 1375 cm^{-1} represents the blending vibration of water molecules. The absorption around 900 cm^{-1} can be assigned to the stretching vibrations of the O–O bond of the peroxo group (–O–O–H). The absorption peak around 640 cm^{-1} can be assigned to the stretching vibration of the Nb–O bond.

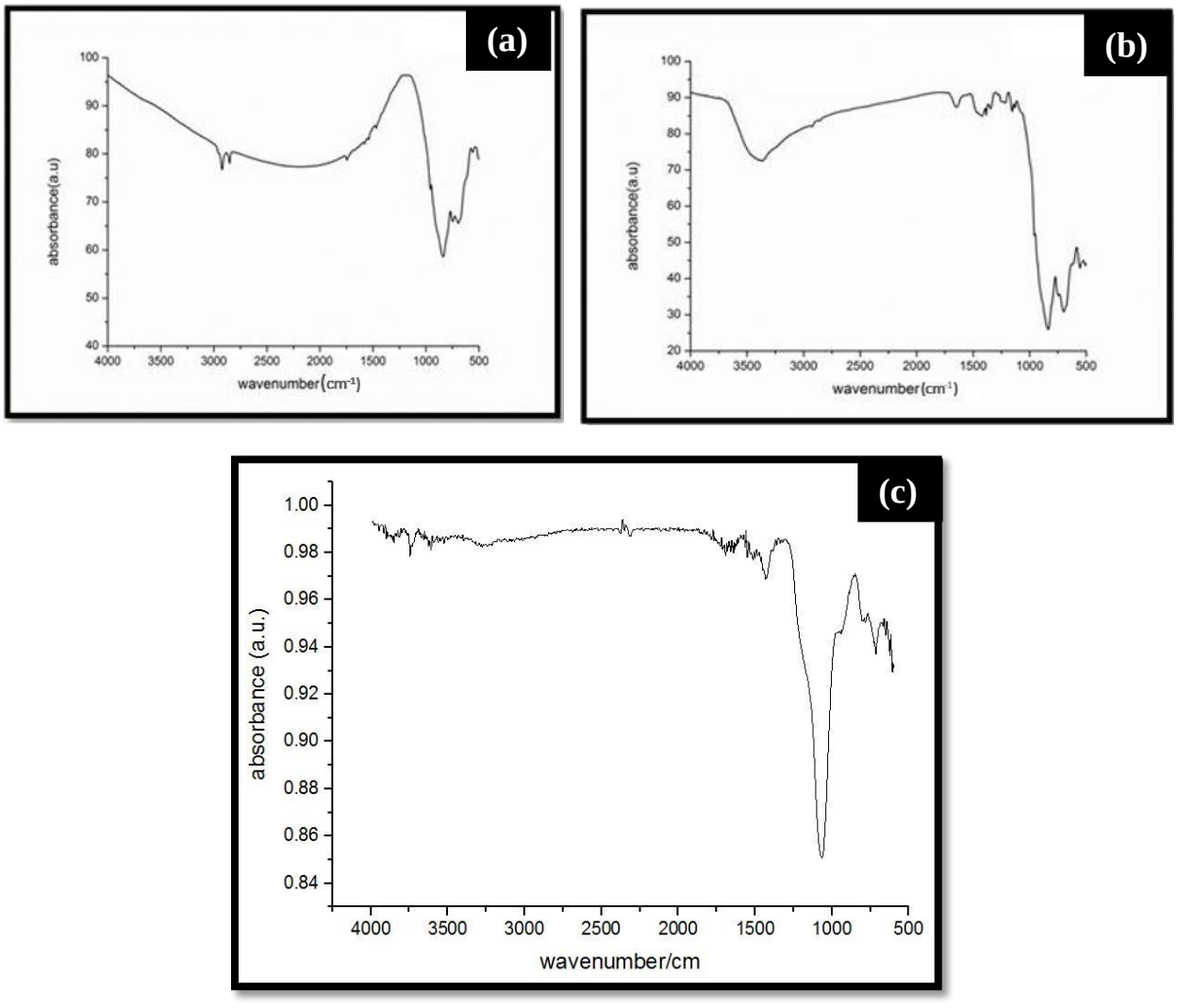


Figure 4. 7: FTIR spectra of (a) Niobium Pentoxide powder (b) Niobium oxide quantum dot (c) Niobium oxide nanoparticle.

4.7 In-Vitro Cytotoxicity assay

The invitro cytotoxicity assay of Niobium oxide nanoparticles in four different concentrations were observed using tissue culture plate (TCP) as control. From the given graph Fig 4.8, it was observed that cells show better viability in Niobium oxide nanoparticles (in mg) than that of the Niobium nanoparticles (in µg). By observing the above data we can conclude that we can use good amount of Niobium oxide nanoparticles for tissue engineering applications due to enhanced cell proliferation much more than the control too.

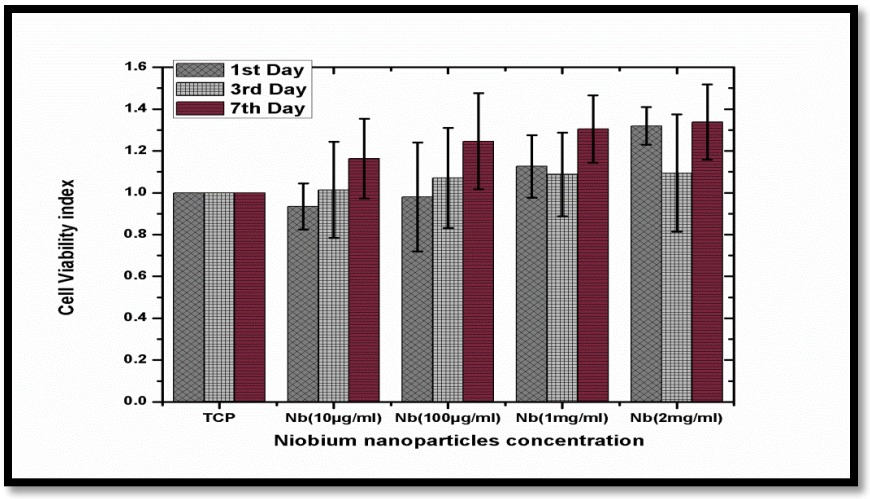


Figure 4. 8: Invitro cytotoxicity assay of Niobium nanoparticles

4.8 Cell Imaging- Hela cells cultured in DMEM containing 4.5g/ glucose, 10% FBS and antibiotics were seeded on a glass cover slip at a density of 10^4 cells per slip and incubated at 37˚C under 5% CO_2 conditions. The niobium oxide nanoparticle solution was added over the cells while maintaining the ratio of niobium oxide nanoparticle solution to media at 1:1.The cells were incubated with the niobium oxide nanoparticle for a period of 3 hours and subsequently visualized under a confocal microscope (Leica TCS). Fig. 4.9 shows the confoca microscopy images of niobium oxide nanopartic es which were taken by exciting different wave ength and observed green and re f uorescence shown in Fig. 4.9(a) and Fig. 4.9(b).

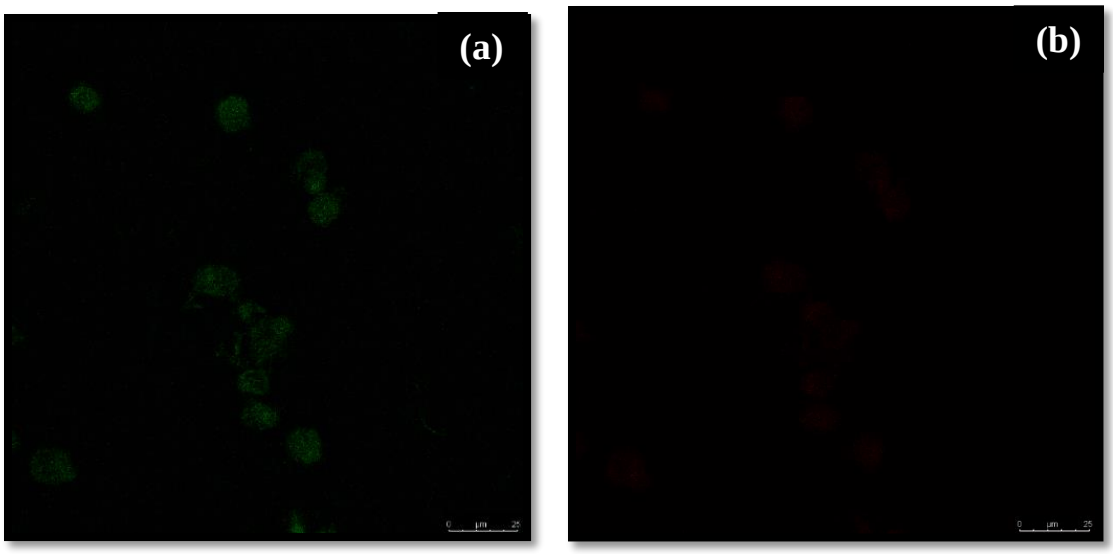


Figure 4. 9: Confocal microscopy images of HeLa cell stained with niobium oxide quantum dot

4.9 Spectrofluorimetry – Spectrofluorimetry of niobium oxide nanoparticle was done by excitation of wavelength 250nm, 260nm, 270nm, 280nm, 290nm and got emission in different region. Fig. 4.10 shows the excitation and emission spectra of niobium oxide nanoparticle.

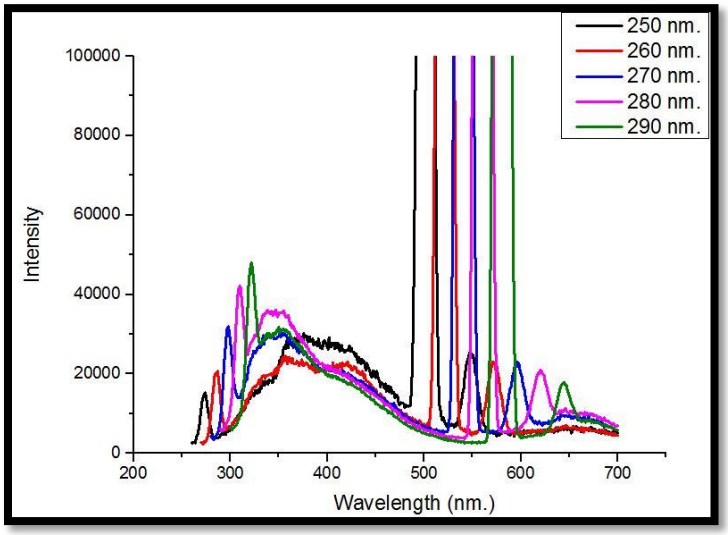


Figure 4. 10: Spectrofluorimetry spectra of niobium oxide nanoparticle

4.10 SUPER CAPACITOR -

Batteries and capacitors are comparable from numerous points of view as they both store and discharge electrical energy. However, some essentia contrasts between them affect their potential applications because of how they work differently. A capacitor comprises of at least two conductive plates isolated by a dielectric. At the point when an electrical current enters the capacitor , the dielectric stops the flow and charge build up and stored in an electrica field between the plates. Every capacitor is intended to have a specific capacitance (energy storage). At the point when a capacitor is associated with an outer circuit, a current will rapidly discharge. In a supercapacitor , there is no dielectric between the plates; rather , there is an e ectrolyte (solid or liquid) and a thin encasing, for example, cardboard or paper. At the point when a current enters to the supercapacitor, ions build on either side of the separator to generate a double layer of charge. Supercapacitors are constrained to low voltages, yet high capacitance , as a high voltage would break down the electrolyte. Different battery sorts are recognized by their chemical makeup. The chemical unit, called the cell, contains three primary parts; a positive terminal called the cathode, negative terminal called the anode, and the electrolyte. The battery charges and discharges through a chemical reaction that produces a voltage. The battery can give consistent and predictab e DC voltage. In rechargeable batteries, the chemical energy that is converted into electricity can be turned around utilizing an outside electrical energy to re-establish the charge.

Development of super capacitor- Electric double layer capacitor was made by using different material as given below.

1. Carboxymethyl cellulose (CMC) – It was used as a binder.
2. Sodium Sulphate and Potassium hydroxide – Both were used as an electrolyte.
3. Charcoal and Niobium oxide nanoparticle – These were used to make electrode.
4. Separator – It was used to separate the two electrodes to avoid the short circuit.

Fig. 4.11 shows the steps involved in making super capacitor.

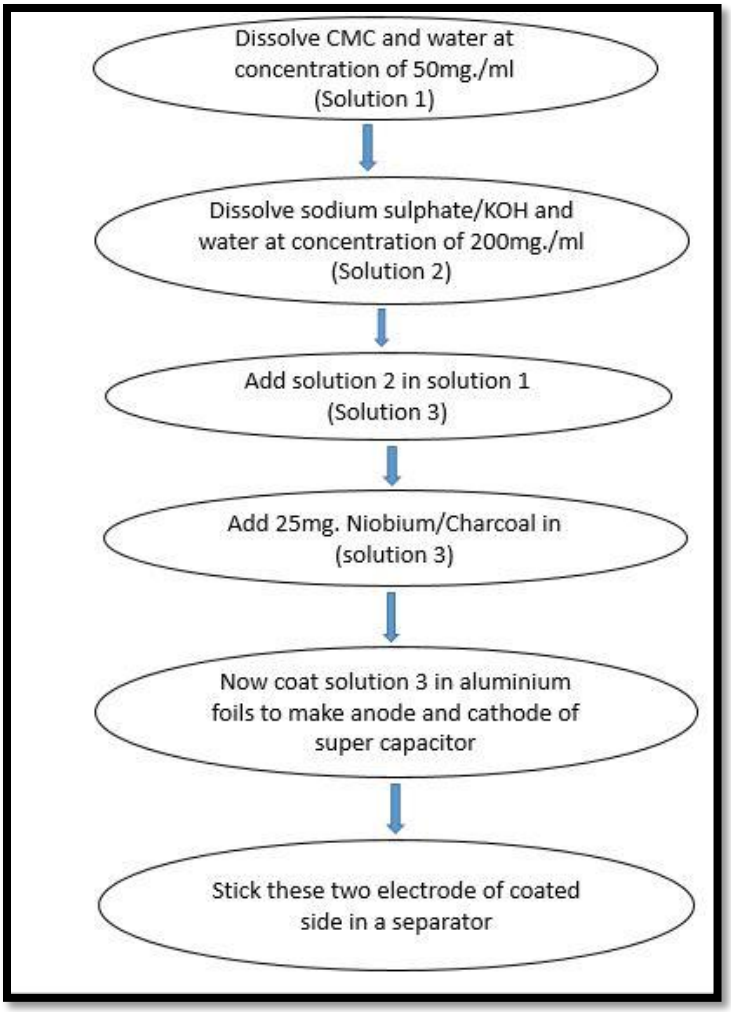


Figure 4. 11:Steps involved in making Supercapacitor

So by following these steps we developed different types of capacitor.

1. Niobium-Charcoal-Sodium Sulphate (4.878 μF) – In this type of supercapacitor we used niobium oxide nanoparticle & Charcoal as electrodes and sodium sulphate as electrolyte. With this configuration we record the capacitor value of 4.878 μF shown in Fig 4.12.

Figure 4. 12 :Niobium-Charcoal-Sodium Sulphate Supercapacitor

2. Niobium-Charcoal-KOH (4.36 μF)- In this type of supercapacitor we used niobium oxide nanoparticle & Charcoal as electrodes and potassium hydroxide as electrolyte. With this configuration we record the capacitor value of 4.36 μF shown in Fig 4.13.

Figure 4. 13 : Niobium-Charcoal-KOH Supercapacitor

3. Charcoal- charcoal- sodium sulphate (2.933 μF) - In this type of supercapacitor we used Charcoal to make both electrodes and sodium sulphate as electrolyte. With this configuration we record the capacitor value of 2.933 μF shown in Fig 4.14.

Figure 4. 14 Charcoal-Charcoal sodium sulphate Supercapacitor

4. Niobium-Niobium-Sodium sulphate (4.915 μF) - In this type of supercapacitor we used Niobium oxide nanoparticle to make both electrodes and sodium sulphate as electrolyte. With this configuration we record the capacitor value of 4.915 μF shown in Fig 4.15.

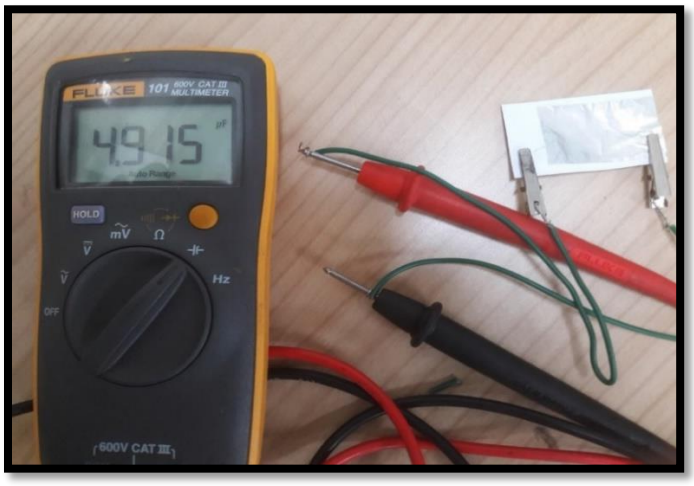


Figure 4. 15 Niobium-Niobium-Sodium sulphate Supercapacitor

5. Charcoal-Charcoal- KOH (0.89 nF) - In this type of supercapacitor we used charcoal to make both electrodes and potassium hydroxide as electrolyte. With this configuration we record the capacitance value of 0.89 nF shown in Fig 4.16.

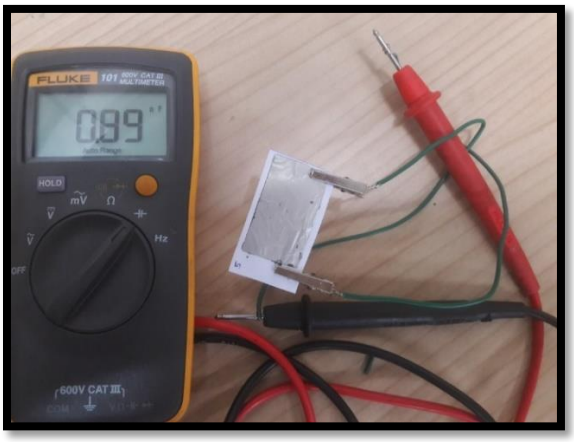


Figure 4. 16 Charcoal-Charcoal- KOH supercapacitor

6. Niobium-Niobium-KOH(0.21 nF) - In this type of supercapacitor we used charcoal to make both electrodes and potassium hydroxide as electrolyte. With this configuration we record the capacitance value of 0.21 nF shown in Fig 4.17.

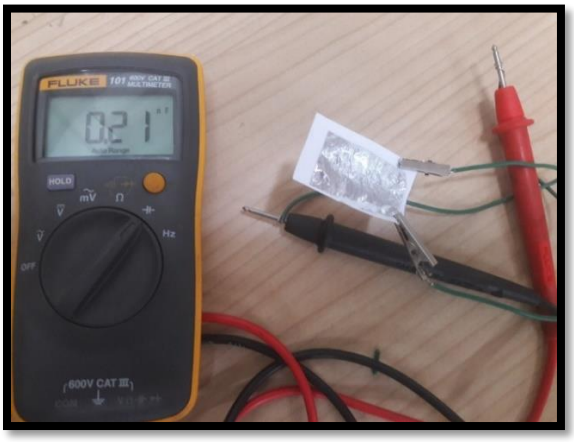


Figure 4. 17 : Niobium-Niobium-KOH Supercapacitor

Chapter 4

Results and Discussion

Part - II

4.11 FE-SEM (Field emission scanning electron microscopy) -

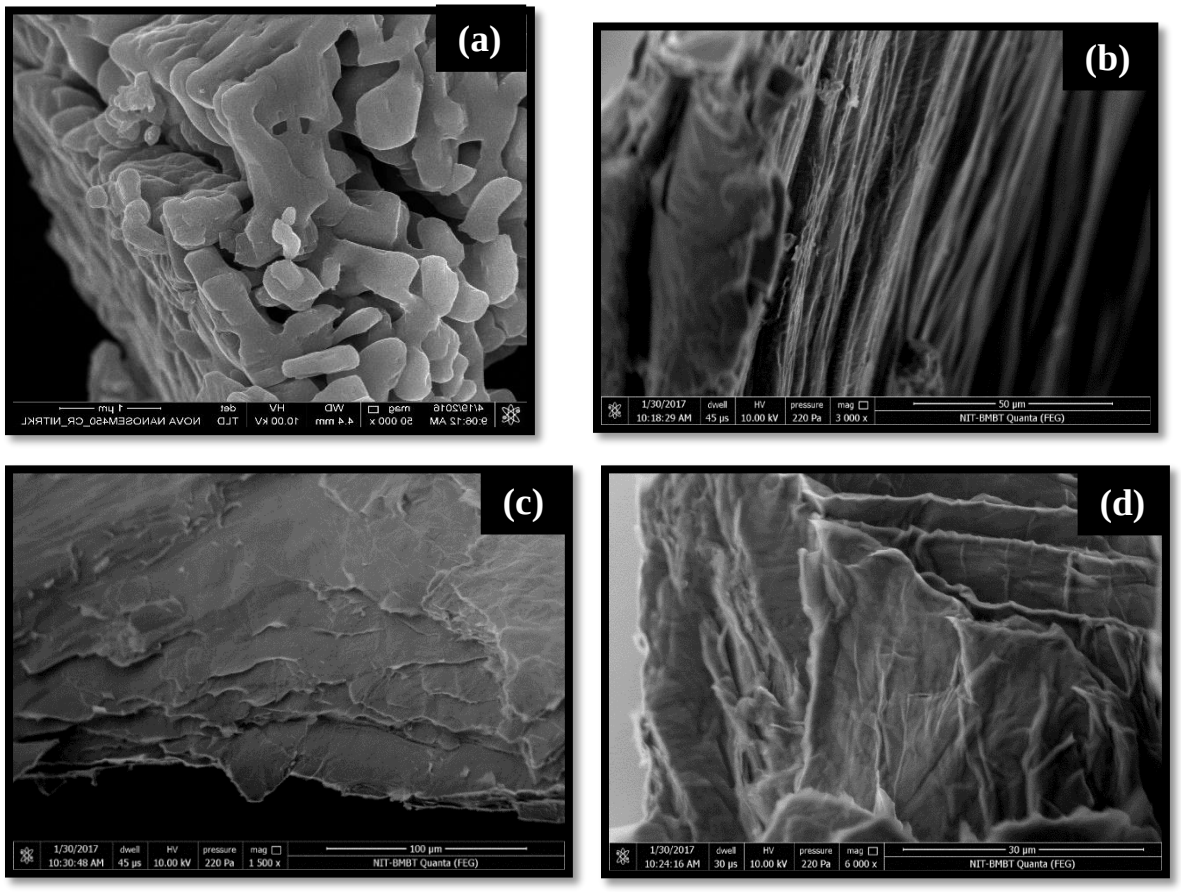


Figure 4. 18: FESEM images of (a) Vanadium Pentoxide powder, (b,c,d) Vanadium layered

Fig. 4.18 (a) shows the FESEM image of vanadium pentoxide powder. Which clearly shows that it constitute large and aggregated particle. Fig. 4.18 (b), (c) & (d) shows the FESEM image of vanadium oxide gel after hydrothermal treatment which shows Vanadium pentoxide aggregated particle was converted into layered Vanadium oxide.

4.12 TEM ((Transmission electron microscope)-

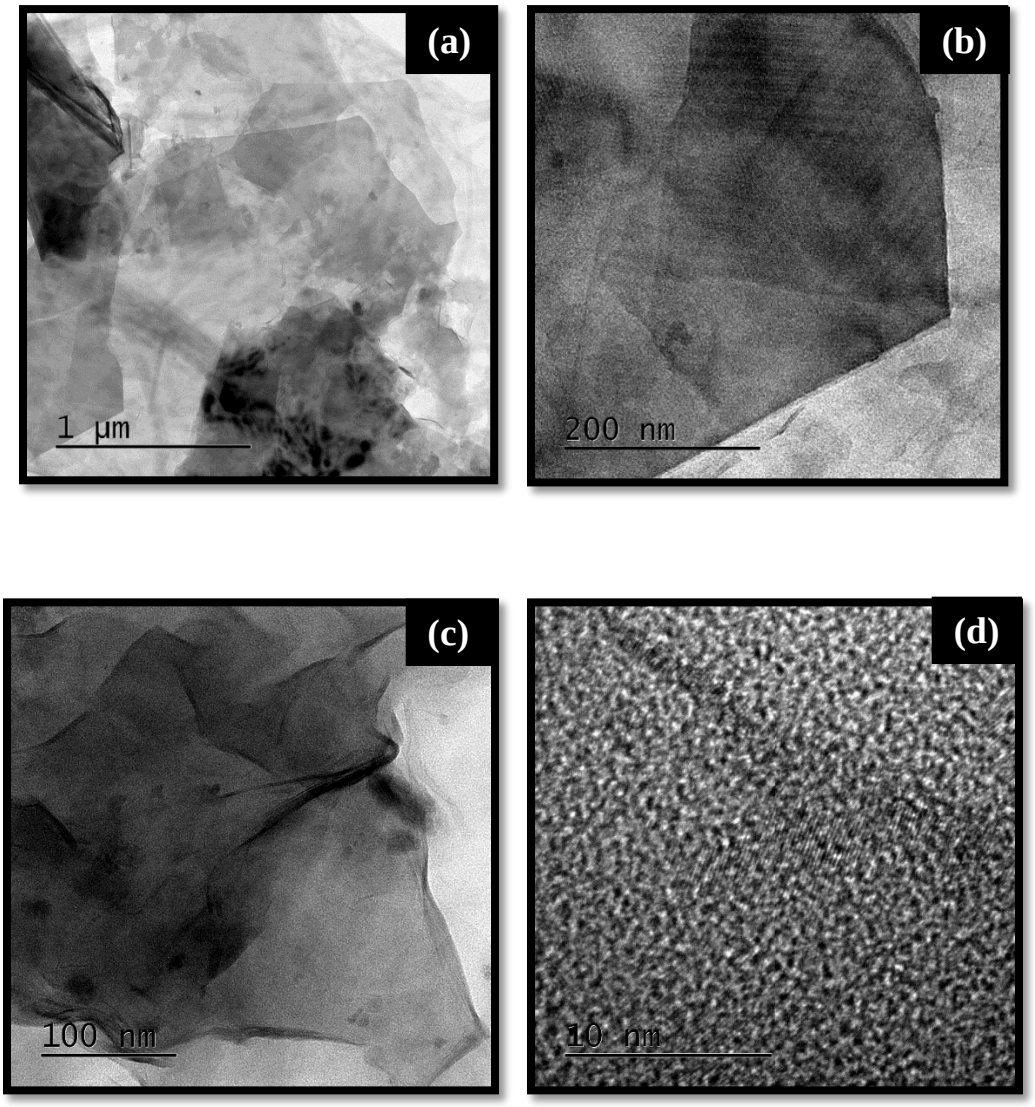


Figure 4. 19: TEM images of Vanadium oxide layer

Fig. 4.19 shows the TEM images of vanadium oxide layer at different magnification. Fig. 4.19 (a) shows that very thin and transparent layered was formed. Fig. 4.19 (b), (c) shows single layer of vanadium oxide. Fringes were also seen which confirms the formation of single layer shown in Fig. 4.19 (d). Thus from the TEM images it is clear that aggregated vanadium oxide particles were converted into very thin, transparent and single layered structure.

4.13 HAADAF (High Angle Angular Dark Field) STEM – The structural distributions of vanadium oxide layer were shown in the Fig. 4.5(a). In STEM mostly layered structures were appeared. Fig. 4.20 (b), (c) shows the elemental position of Oxygen and Vanadium in vanadium oxide layer shown in STEM image Fig. 4.5(a). The layered structure of vanadium oxide contains mainly oxygen and vanadium.

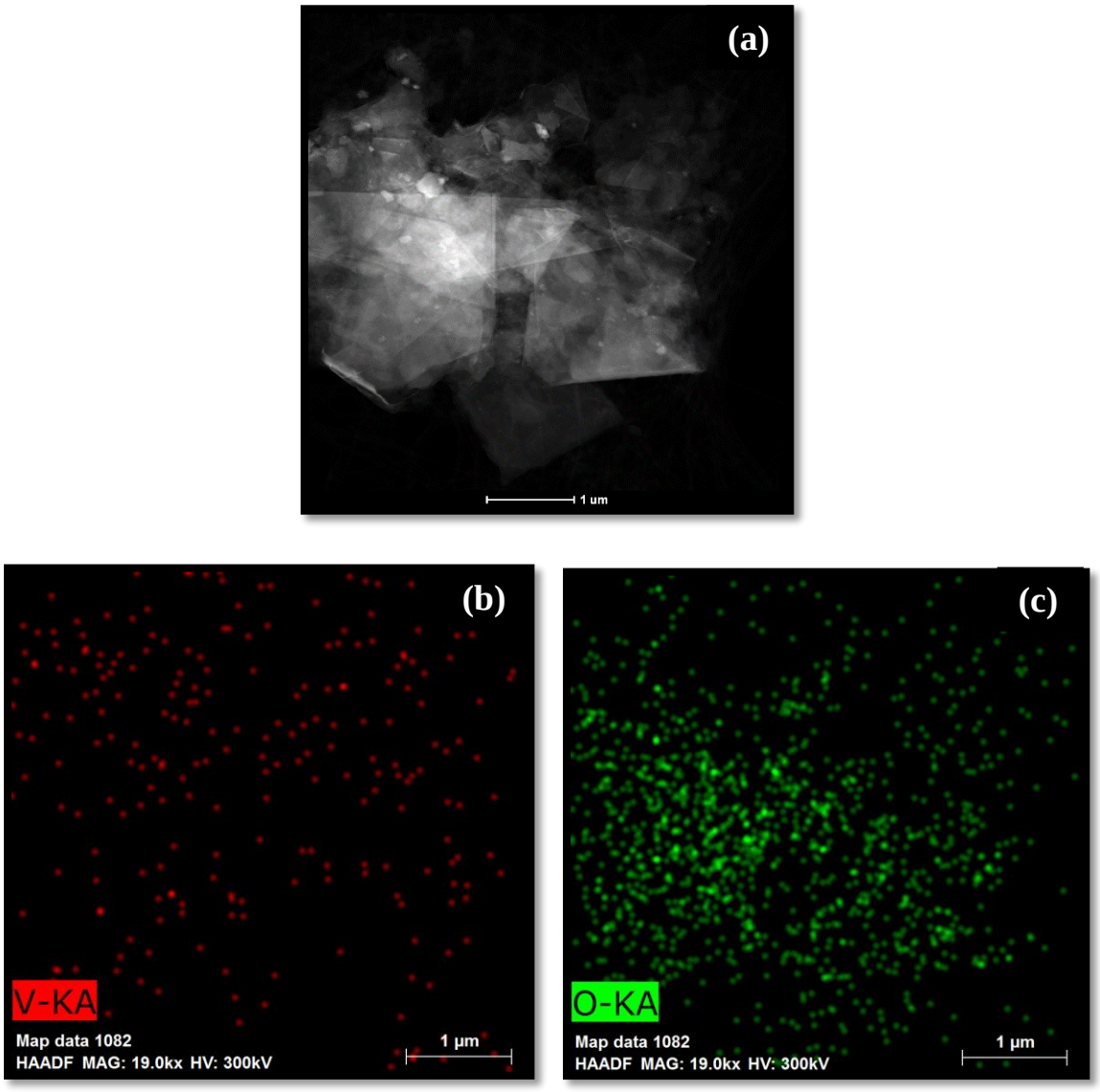


Figure 4. 20: HAADF image of Vanadium oxide layer

4.14 XRD (X-ray Diffraction) - XRD pattern of vanadium pentoxide powder is shown in Fig. 4.12(a) which shows crystalline structure. Fig. 4.21 (b) shows the XRD pattern of H2O2 treated vanadium oxide layer which shows amorphous like structure. Fig. 4.21 (c) shows the XRD pattern of H2O treated vanadium oxide layer which shows highly crystalline structure with high intensity as shown in Fig. 4.21(c).

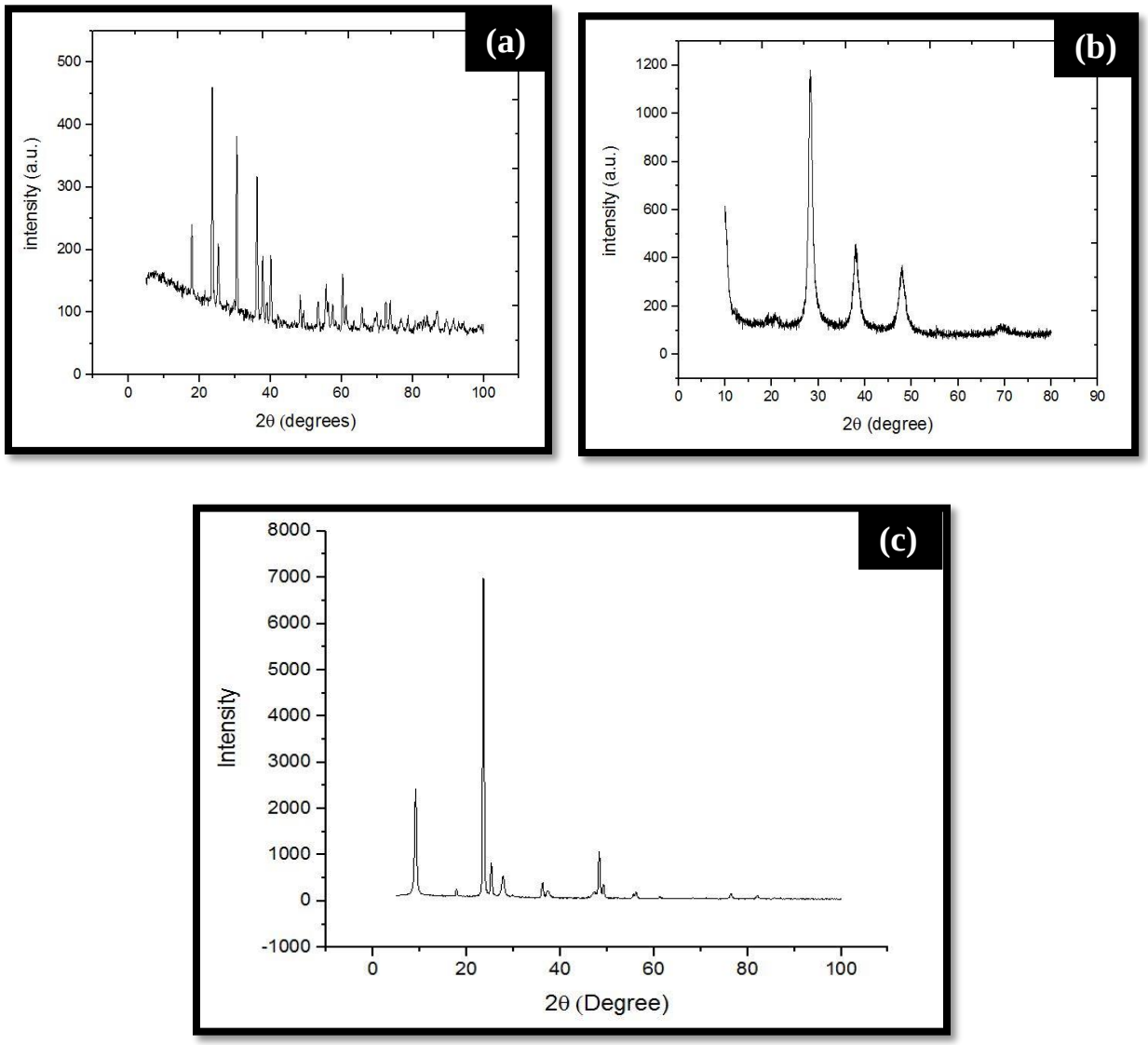


Figure 4. 21: XRD Pattern of Vanadium pentoxide powder(a) vanadium oxide layer H_2O_2(b) vanadium oxide layer H_2O(c)

4.15 Cell Imaging- HeLa cells were treated with the vanadium quantum dots such that the ratio of vanadium quantum dot solution to media was maintained at 1:1.The cells were incubated with the vanadium quantum dots for a period of 3 hours and subsequently visualised under a fluorescent microscope. The uptake of the vanadium quantum dots by the cells was clearly observed from the blue and green colored fluorescence emitted by them as shown in Fig.4.22.

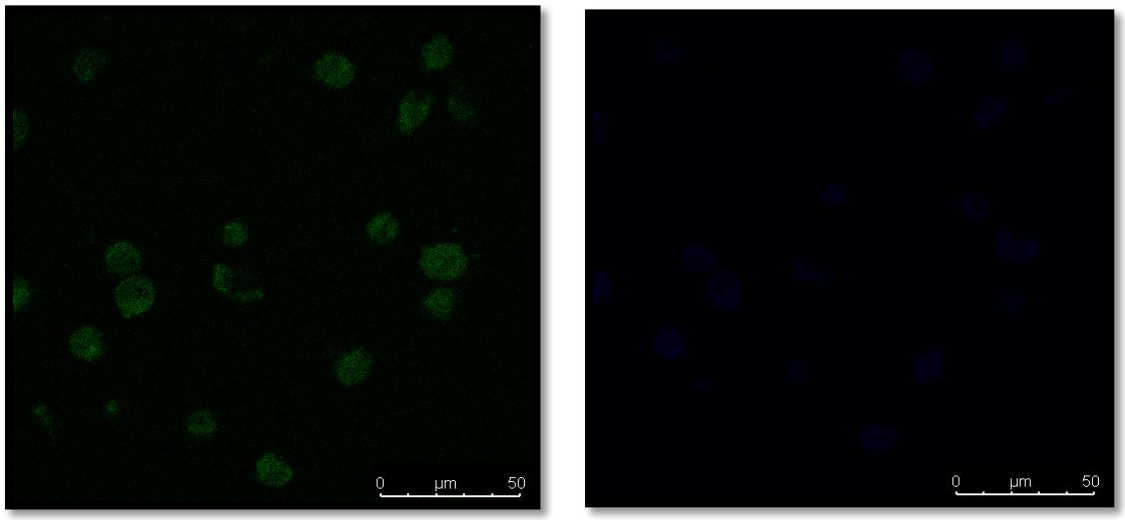


Figure 4. 22 Confocal microscopy images of HeLa cell stained with vanadium oxide quantum dot

Chapter 5

Conclusion

Niobium nanoparticles, quantum dot and vanadium layer, vanadium quantum dot are successfully synthesized by hydrothermal treatment followed by sol-gel method. Crystalline niobium oxide nanoparticles were synthesized by heating niobic acid precipitate with hydrogen peroxide and niobic acid nanoparticles were synthesized by heating niobic acid precipitate. Niobium acid precipitate was obtained by using ammonia solution. So basically we can say that the structure, morphology and surface area of niobic acid nanoparticles is regulated by the ammonia solution. After hydrothermal treatment, the bigger size nanoparticle become smaller due to hydrogen peroxide. Vanadium pentoxide treated with hydrogen peroxide and after hydrothermal treatment converted into layered structure and vanadium quantum dot. The morphology and structure of vanadium oxide layer was thin, transparent and single layer. The synthesized niobium oxide nanoparticle, quantum dot showed the application in live cell imaging and supercapacitor. The vanadium oxide quantum dot also showed the application in cell imaging. The structure , morphology and size of the vanadium quantum dot depends upon the molar ratio of vanadium used in hydrothermal treatment and also time duration of hydrothermal treatment play important role during the synthesis of vanadium quantum dot and vanadium layer. Cell cytotoxicity assay showed that within increase in concentration of niobium oxide nanoparticles the cell proliferation rate or cell viability index increases. From the above result we can conclude that both quantum dots are biocompatible and we can use these nanoparticles as a biomaterial, cell imaging & storage devices etc.

References

1. Access, Y. (2016). Your Access to the most cited sources in Chemistry ends in 1 day, 1–2. http://doi.org/10.1021/nl403674a
2. Chen, Y., Yang, G., Zhang, Z., Yang, X., Hou, W., & Jie, J. (2016). Polyaniline - intercalated layered vanadium oxide nanocomposites — One - pot hydrothermal synthesis and application in lithium battery †, 1–19. http://doi.org/10.1039/C0NR00246A
3. Chen, Z., Yin, J., Zhou, Y., Zhang, Y., Song, L., Song, M., … Al, C. E. T. (2012). Dual Enzyme-like Activities of Iron Oxide Nanoparticles and Their Implication for Diminishing Cytotoxicity, (5), 4001–4012.
4. Farahi, F. (n.d.). 1nanoparticles.pdf.
5. Guo, X., Wang, Y., Wu, F., Ni, Y., & Kokot, S. (2015). A colorimetric method of analysis for trace amounts of hydrogen peroxide with the use of the nano - properties of molybdenum disulfide †, 1–17. http://doi.org/10.1039/C4AN01950D
6. Kianfar, E. (2015). Production and Identification of Vanadium Oxide Nanotubes, *8*(May), 455–464.
7. Koohestani, B., Tajdari, M., & Ebrahimi, F. (2015). Synthesis of Vanadium Oxide Nanostructures Using Hydrothermal Method and the Utilizes of, *5*(1), 2143–2150.
8. Krumeich, F., Muhr, H. J., Niederberger, M., Bieri, F., Schnyder, B., & Nesper, R. (1999). Morphology and topochemical reactions of novel vanadium oxide nanotubes. *Journal of the American Chemical Society*, *121*(36), 8324–8331. http://doi.org/10.1021/ja991085a
9. Li, X. (2012). Improved Pyrogallol Autoxidation Method: A Reliable and Cheap Superoxide-Scavenging Assay Suitable for All Antioxidants.
10. Livage, J. (2010). Hydrothermal synthesis of nanostructured vanadium oxides. *Materials*, *3*(8), 4175–4195. http://doi.org/10.3390/ma3084175
11. Muhr, B. H., Krumeich, F., Schönholzer, U. P., Bieri, F., Niederberger, M., Gauckler, L. J., & Nesper, R. (2000). Vanadium Oxide NanotubesÐA New Flexible Vanadate Nanophase **. *Advanced Materials*, *12*(3), 231–234. http://doi.org/10.1002/(SICI)1521-4095(200002)12:3<231::AID-ADMA231>3.0.CO;2-D
12. Naguib, M., Halim, J., Lu, J., Cook, K. M., Hultman, L., Gogotsi, Y., & Michel, W. (2016). New Two - Dimensional Niobium and Vanadium Carbides as Promising Materials for Li - Ion Batteries Partners, 1–2. http://doi.org/10.1021/ja405735d
13. Niederberger, M., Muhr, H. J., Krumeich, F., Bieri, F., Gunther, D., & Nesper, R. (2000). Low-Cost Synthesis of Vanadium Oxide Nanotubes via Two Novel Non-Alkoxide Routes. *Chemistry of Materials*, *12*(7), 1995–2000. http://doi.org/Doi 10.1021/Cm001028c
14. Pinna, N., Antonietti, M., & Niederberger, M. (2004). A novel nonaqueous route to V2O3

and Nb2O5 nanocrystals. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *250*(April), 211–213. http://doi.org/10.1016/j.colsurfa.2004.04.078

15. Saha, K., Agasti, S. S., Kim, C., Li, X., & Rotello, V. M. (2012). Gold nanoparticles in chemical and biological sensing. *Chemical Reviews*, *112*(5), 2739–2779. http://doi.org/10.1021/cr2001178
16. Spahr, M. E., Bitterli, P., Nesper, R., Müller, M., Krumeich, F., & Nissen, H. U. (1998). Redox-active nanotubes of vanadium oxide. *Angewandte Chemie - International Edition*, *37*(9), 1263–1265. http://doi.org/10.1002/(SICI)1521-3773(19980518)37:9<1263::AID-ANIE1263>3.0.CO;2-R
17. Uecker, H., & Sorge, A. (2006). Quantum Dots, 19. http://doi.org/10.1007/978-3-642-44910-9
18. Uekawa, N., Kudo, T., Mori, F., Wu, Y. J., & Kakegawa, K. (2003). Low-temperature synthesis of niobium oxide nanoparticles from peroxo niobic acid sol. *Journal of Colloid and Interface Science*, *264*(2), 378–384. http://doi.org/10.1016/S0021-9797(03)00460-0
19. Wu, M., Zhang, X., Gao, S., Cheng, X., Rong, Z., & Xu, Y. (2016). Construction of monodisperse vanadium pentoxide hollow spheres via a facile route and triethylamine sensing property †, 1–16. http://doi.org/10.1039/C3CE41471J
20. Zhou, Y., Qiu, Z., L??, M., Zhang, A., & Ma, Q. (2008). Preparation and characterization of porous Nb2O5 nanoparticles. *Materials Research Bulletin*, *43*(6), 1363–1368. http://doi.org/10.1016/j.materresbull.2007.06.053